Final research report to AOARD in 2006

Title of Research Project: **High Density Data Storage Systems by DNA Complexes and Nano-particles from DNA Hybrid Materials**

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SUMMARY

Research 1 Novel DNA-lipid films derived from alanine

Chiral lipids were synthesized from alanine having C10 alcohol unit as an ester group and DNA hybrid complexes were prepared from these chiral lipids. The as-cast DNA-L-alanine C10 complex film showed a self-assembled structure which was enhanced by water, while no self assembled structure was observed for the DNA-D-alanine C10 complex film.

Research 2 *In-situ* Intercalation of Phtharocyanine dye PC with DNA and Polyamine Complex

In-situ film formation of DNA film with polyamine complexes was carried out in the presence of Cu-PC in water and a blue film was obtained. Refractive indices of the DNA-Cu-PC films changed before and after UV irradiation and an erasable memory device is possible.

Research 3 Nano-particles derived from DNA-polymer Hybride Materials Containing Optical Dyes

Optical properties of DNA-containing particle and film were compared in terms of threshold values of half-width narrowing spectra and DNA-containing particles showed much narrowing of spectrum width with a threshold value of 1 mJ/cm^2, while the narrowing range of the half-width of the DNA-containing film was around 40 nm and no threshold value was observed. DNA-containing particles enhanced fluorescence light much better than film, possibly owing to light enhancement by a resonance effect within the nano-particles

Research 4 Design and demonstration of high density data storage system.

A grating type film wave guide was designed for the high density data storage. Results on light reflection by using the DNA grid-type device showed a very sharp reflection at 1560.5nm which was expected by the simulation. This result suggests practical applications of the DNA optical device.

Workplan in 2006 and 2007: Attached

Research 1 Novel DNA-lipid films derived from alanine

Deoxyribonucleic acid (DNA) which contains gene information of all living things is a water-soluble polymers with a very high molecular weight of over billions. DNA molecules is an

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14. ABSTRACT
The work focused on (1) syntheses and characterization of Novel DNA-lipid films derived from alanine, (2) In-situ Intercalation of Phtharocyanine dye (PC) into DNA and Polyamine Complex, (3) syntheses and characterization of Nano-particles derived from DNA-polymer Hybrid Materials Containing Optical Dyes, and (4) demonstration of high density data storage system using DNA complex.

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anionic polyphosphate with sodium cation as a counter ion (DNA-Na), which can be replaced by lipids having quarternized ammonium cation to form DNA-lipid hybrid complexes. The ion exchange reaction of sodium cation of DNA takes place by simply mixing an aqueous solution of DNA-Na with an aqueous solution of excess amount (more than twice molar ratios) of lipids at ambient temperature and a DNA-lipid hybrid complex is precipitated out of the aqueous solution. The DNA-lipid hybrid complexes become insoluble in water and become soluble in polar organic solvents such as alcohols, and transparent films of DNA-lipid hybrid complex are obtained by a simple solvent casting method[1]. Thus, various applications of DNA have been developing in wide areas of photonic or electronic devices[2-6].

Physical properties of the DNA-lipid complexes are strongly dependent on kinds of lipid in terms of number of alkyl chain length of lipids[7]. It was reported[8] that when a cationic lipid having chiral structure such as cholesteryl 3β-*N*-((dimethylamino)ethy)carbamate (DC-Chol) was used to form a binary complex of DNA and cationic lipid□a self-assembled structure of DNA-DC-Chol complex was obtained. More simple chiral lipids may induce to construct a strong self-assembled structure of DNA hybrid film.

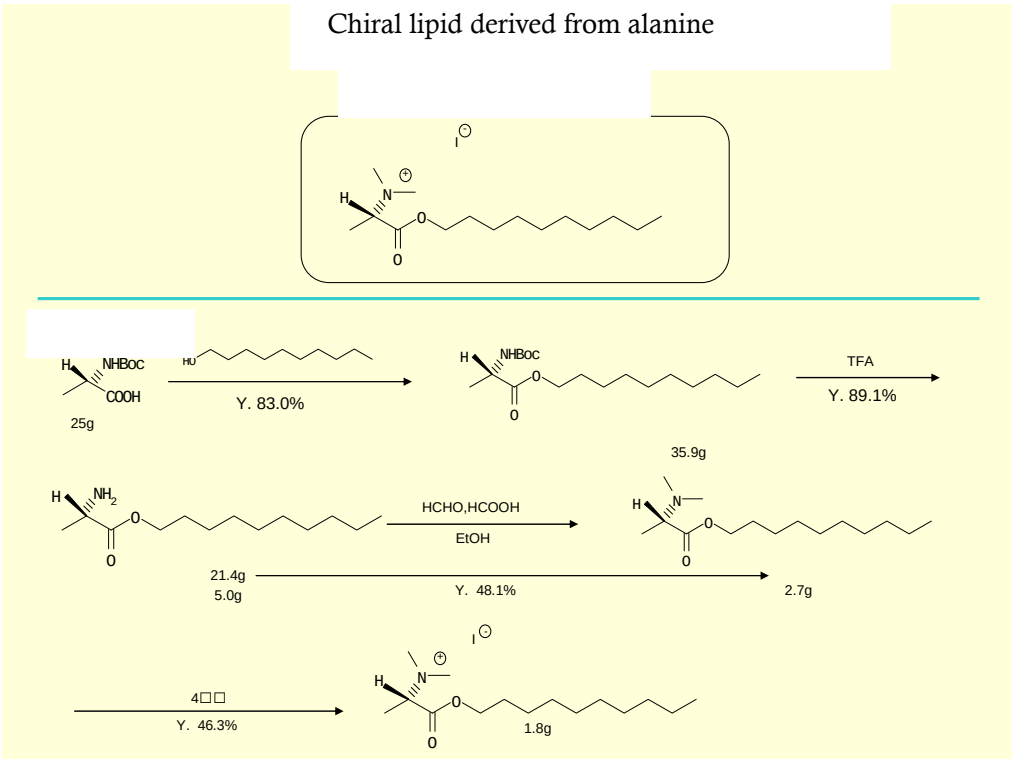
The oriented structure of DNA molecules is very important for photonic properties such as second harmonic generation (SHG) of optical dyes which are intercalated into the double helix of DNA molecules. Magnetic orientation of DNA molecules was tried before by using a high power magnetic field which was applied to DNA-lipid solutions while they are evaporating. However, the oriented structure of DNA molecules was not sufficient enough to induce a strong SHG. Therefore, it is highly recommendable to induce self-assembled structure of DNA molecules by introducing a chiral lipid with DNA molecules.

This paper describes characteristic features of DNA-chiral lipid complex films which were derived from alanine.

EXPERIMENTAL

1. Preparation of chiral lipids from alanine

Chiral lipids with alkyl group of C=10 as an ester group of L-alanine (abbrebiated as L-alanine C10) were synthesized by following synthetic route starting alanine as shown in scheme 1 where yields of each synthetic steps were also shown. L-,D-, and racemic lipids were synthesized by the same synthetic route. When long alkyl alcohols of more than C=16 were used to prepare alanine lipids, lipids were not soluble in water so that alcohol of C=10 was used.



Scheme 1 Synthetic route

2. Preparation of DNA-lipid complex and films

Pure DNA derived from Salmon was dissolved an amount of 1g in 100 ml water. Chiral lipids derived from L-, D- or racemic alanine were dissolved in an amount of each 1g in 100 ml of water. The DNA-alanine-derived lipid complexes were obtained by adding dropwisely the aqueous solution of these alanine-derived lipids into the aqueous solution of DNA, followed by filtering and drying in vacuum at ambient temperature. Films of the DNA-alanine-derived lipid complexes were obtained by casting solutions of the DNA-lipid complexes in hexafluroisopropanol (1□□100 ml) onto a Teflon-coated plate, followed by drying in vacuum to obtain transparent films. The DNA-lipid complexes were soluble in ethanol right after the preparation of the complexes, while they became insoluble in any other organic solvents except hexafluroroisopropanol, by standing possibly owing to crystallization.

3. Physical Properties

Tensile strength and elongation of films were measured by Toyo Tensiron instrument at ambient temperature after drying the films in vacuum. Water absorption behaviors of the films were determined by keeping the films under various relative humidity at ambient temperature for 2 days, followed by measuring weight increases of the films. Microscopic pictures of the films were observed by a cross-polarized microscope. Surface structures of the films were observed by

a JEOL scanning microscope.

RESULTS AND DISCUSSION

Figure 1 shows stress-strain curves of DNA-lipid films derived from DNA and alanine C10, in comparison with film properties of DNA-CTMA (cetyltrimethylammonium chloride) and DNA-EF132 (fluoroalkylammonium chloride, structure is unknown) complexes. It can be clearly pointed out in Figure 1 that the film derived from DNA-L-alanine C10 indicated a characteristic feature of mechanical properties in terms of strength and elongation, which were almost more than 10 times higher in strength and elongation that those of DNA-CTMA comlex film.. On the other hand, the films from DNA-D-alanine C10 and also DNA-racemic alanine C10 showed almost similar mechanical properties as the DNA-CTMA complex film. The DNA-EF132 films indicated a longer breaking elongation of 25 mm because of slippery fluorinated alky chain than that of DNA-CTMA, DNA-D- or racemic alanine C10 films. However, the elongation of the DNA-L-alanine C10 film was twice as much as that of DNA-EF132 (fluorinated alkyl lipid) film.

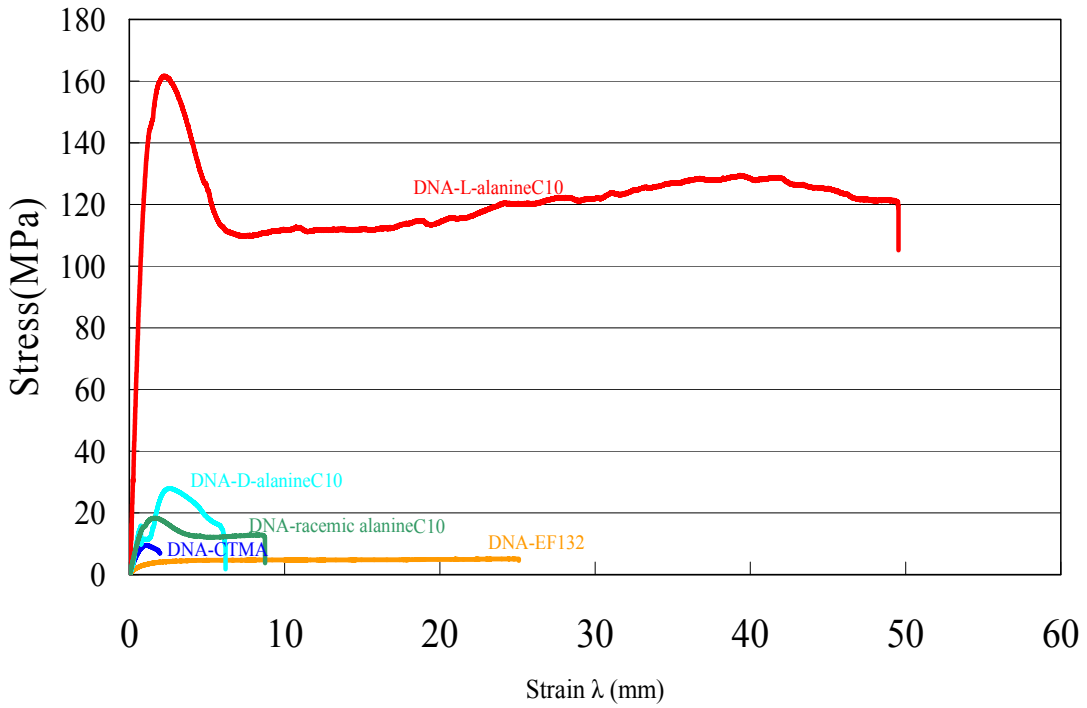


Figure 1 Stress-Strain Curves of DNA-lipid complex films

Water absorption behaviors of various DNA-lipid films were summarized in Figure 2, where it is seen that DNA-L-alanine C10 film showed much less water absorption than other DNA-lipid complex films, especially under high relative humidity of more than 80%. DNA-lipid complexes

containing one single alky chain of C=12,14 and 16 showed a high water absorption at high relative humidity of more than 80%, while water absorption values decreased with increasing carbon number of lipids because of hydrophobic increase owing to long alkyl chains. Water absorptions of DNA-D-alanine C10 or DNA-racemic alanine C10 were less than those of DNA-single alkyl chain lipid of C=12.

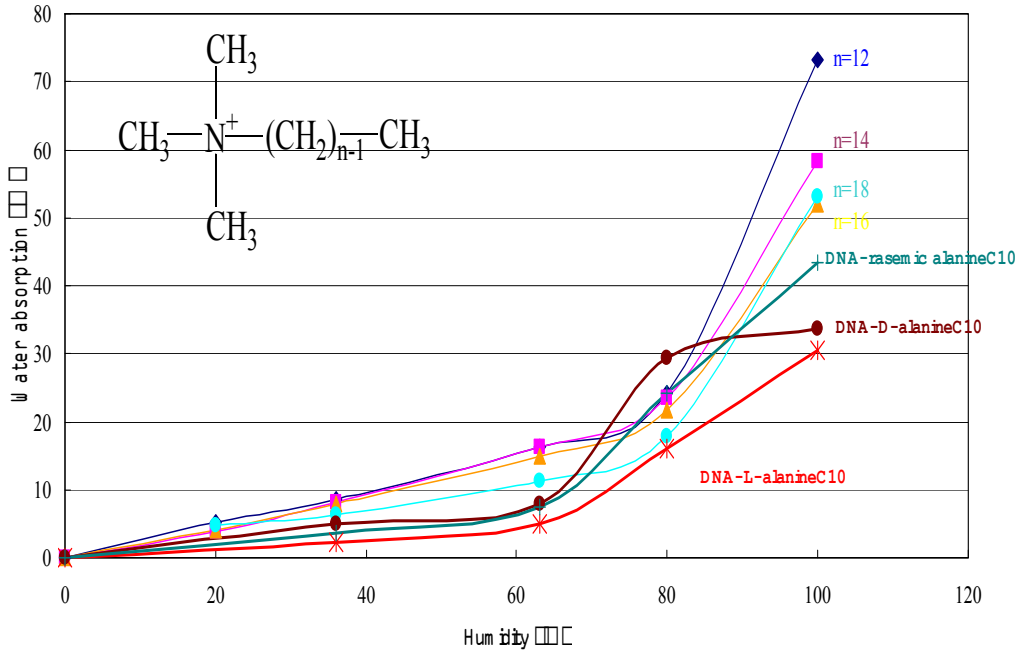
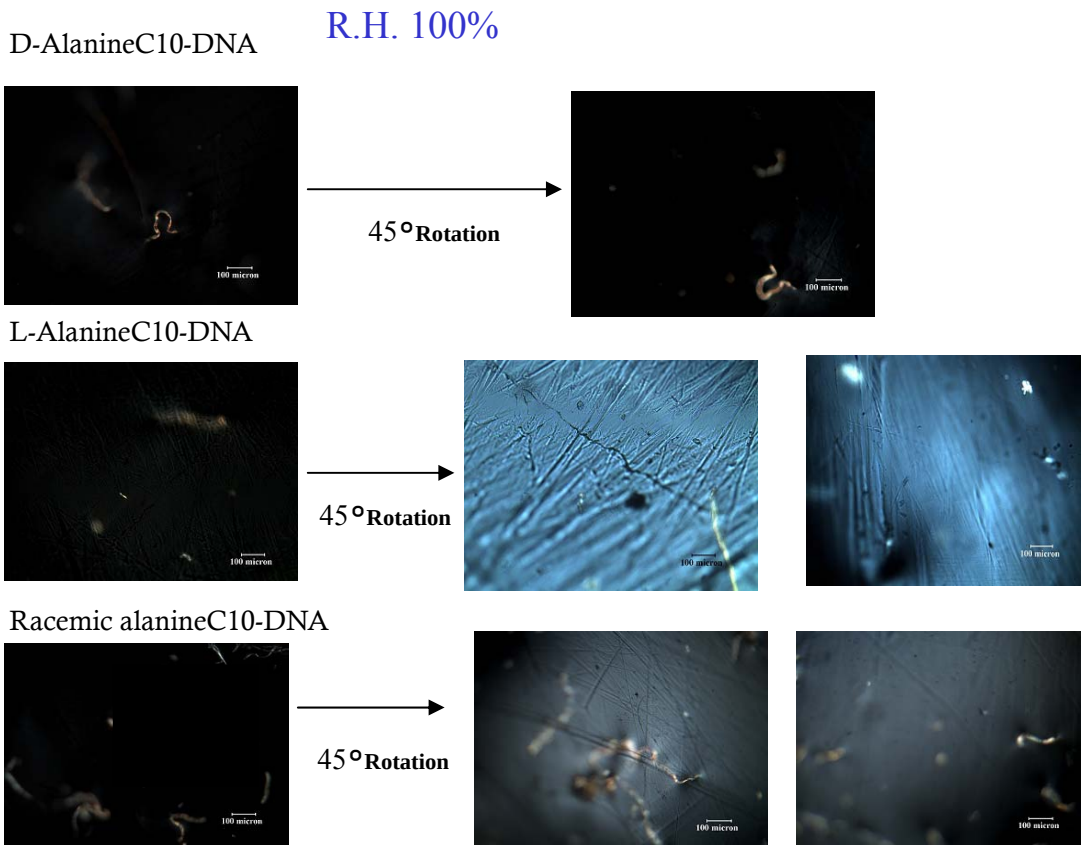


Figure 2 Water absorption behaviors of DNA-lipid complex films

Figure 3 indicates cross-polarized microscopic pictures of DNA-L-alanine C10, DNA-D-alanine C10 and DNA-racemic alanine C10 films under 100% relative humidity at ambient temperature, respectively. When a polarizer was rotated at 45° angle, no change of the film picture for the DNA-D-alanine C10 was observed, showing only a dark background. On the other hand, the DNA-L-alanine C10 film showed a clear bright picture by rotating a polarizer at 45° angle, indicating an oriented structure of DNA-L-alanine C10 molecules. DNA-racemic alanine C10 film showed a little bright picture by rotating a polarizer at 45° angle. It is quite interesting that the DNA-D-alanine C10 did not show an oriented structure and DNA-racemic alanine C10 showed a little oriented structure under a cross-polarized microscope. As-cast film of the DNA-L-alanine C10 showed a clear oriented structure as shown in Figure 4, while a dark picture was observed after drying the film at 0 % relative humidity. However, gradual increases

in relative humidity from 61%, 80% to 100% resulted in a recovery of an oriented structure of the film, as shown in Figure 4. Drying process may cause a randomization of oriented structure of DNA-L-alanine C10 molecules by heating , while increasing water content in the film may stimulate the reconstruction of a self-assembled structure of the DNA-L-alanine molecules, possibly by a plasticizing effect of water.



Figures 3 Cross-polarized pictures of as-cast DNA-alanine C10 films at 100%RH

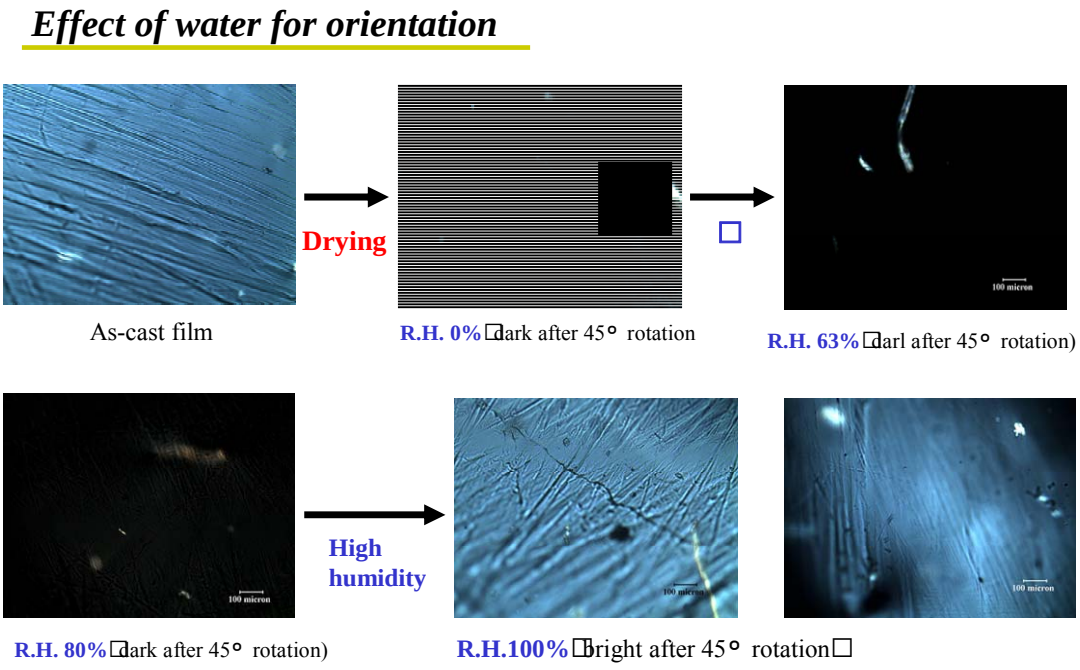


Figure 4 Effect of water for reorientation of DNA-L-alanineC10 complex film

As-cast film of DNA-L-alanineC10 exhibited an oriented structure after elongation of 4 times, while it showed the same oriented structure by keeping the film at 100%RH. It is quite interesting that the DNA-L-alanineC10 complex film showed a self-assembled and oriented structure which would be useful for the orientation of optical dyes for second harmonic generation of light. This expectation will lead to next research target.

DNA-L-alanineC10

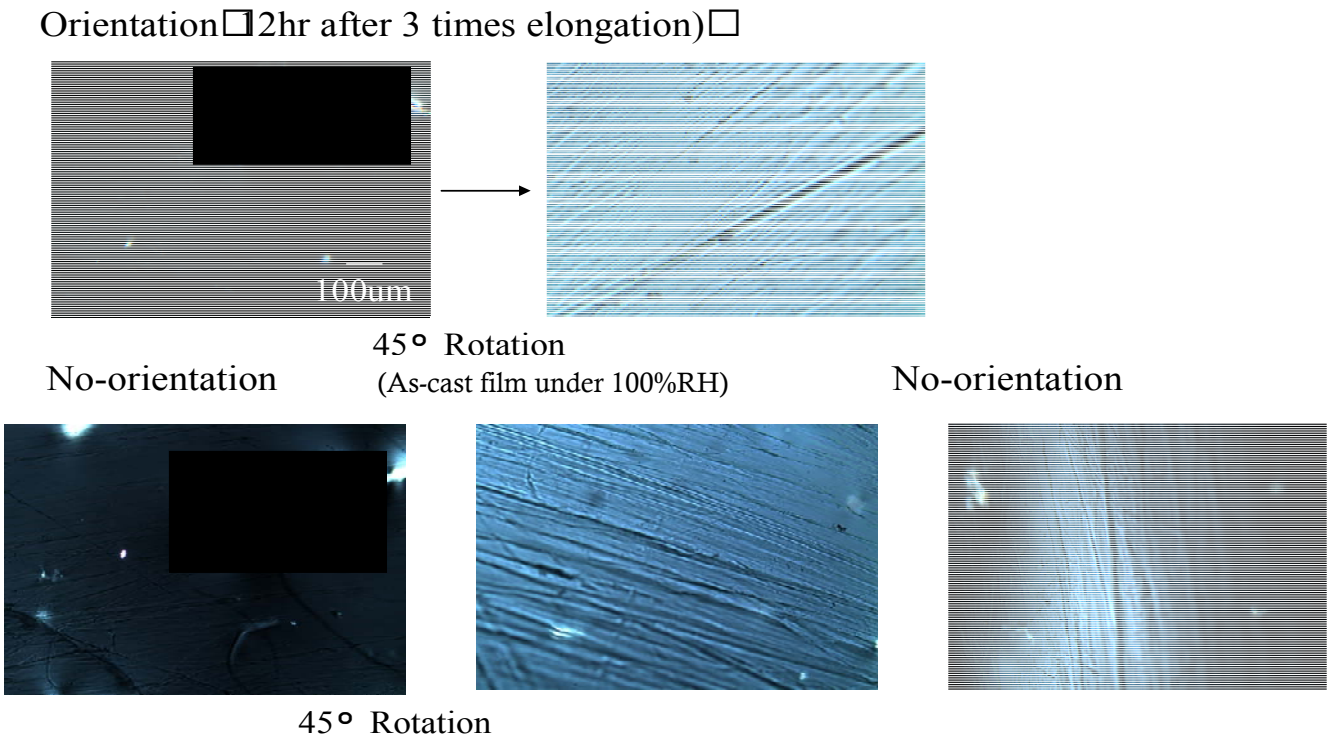


Figure 5 Oriented structure of DNA-L-alanineC10 film after elongation and exposure at 100%RH

Surface structures of these as-cast DNA-alanine C10 complex films were observed by a scanning probe microscope (SPM) and SPM pictures are summarized in Figure 6 which showed each three different magnifying scales. The DNA-D-alanine C10 film showed a smooth surface without any particular patterns, while the DNA-L-alanine C10 film exhibited an uneven surface with a regular interval pattern. The DNA-racemic alanine C10 film also showed the similar uneven surface with a regular interval pattern as the DNA-L-alanine C10 film, as shown in Figure 6. These regular uneven pattern of these films may be ascribed to an oriented structure of the DNA-L-alanine C10 molecules. The lower water absorption behaviors of the DNA-L-alanine C10 complex film which are shown in Figure 2 can be explained by the oriented structure to increase crystallization which would decrease the water absorption.

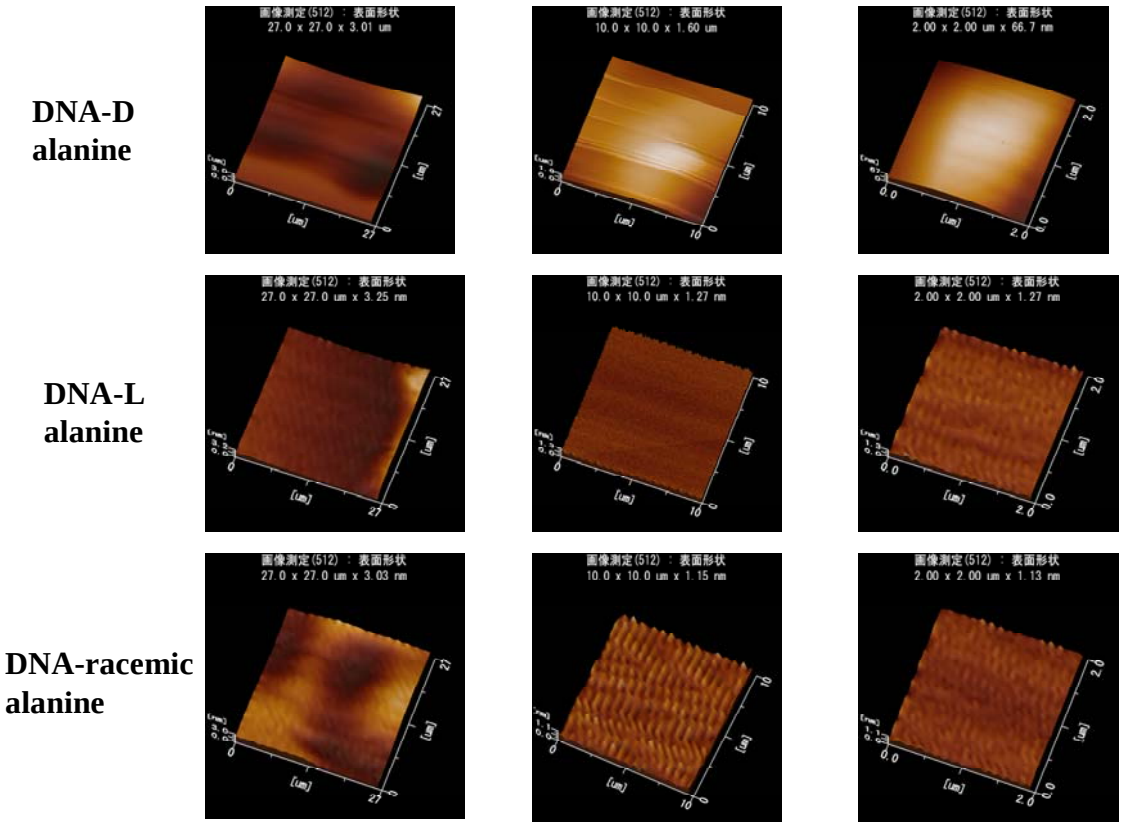


Figure 5　SPM pictures of surface structures of DNA-alanine C10 complex films

It is not clear why the DNA-D-alanine C10 which is an other side of optical isomers of the DNA-L-alanine C10, did not showed a self-assembled oriented structure. The DNA-racemic alanine C10 indicated a little self-assembled structure which may be ascribed to a half amount of L-alanine units. The enhanced self-assembled structure of the DNA-L-alanine C10 molecules may be related with left-hand side helical directions of DNA molecules as the L-alanine unit is attached to DNA molecules as side chains ,and D-alanine unit may not fit with the helical directions of DNA molecules to organize a self-assembled structure. More detailed studies may be needed in terms of X-ray analyses.

CONCLUSION

The as-cast DNA-L-alanine C10 complex film showed a self-assembled structure which was enhanced by water, while no self assembled structure was observed for the DNA-D-alanine C10 complex film.

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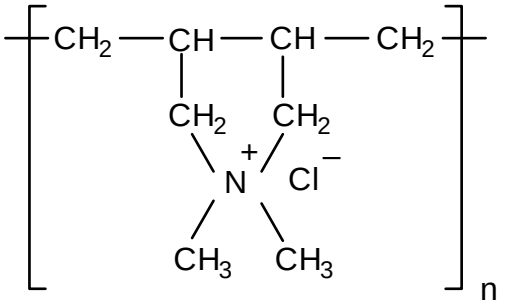
Research 2 *In-situ* Intercalation of Phtharocyanine dye with DNA

Intercalation of DNA by various photochromic dyes into double helix of DNA induced to enhance and amplify light owing to electron-electron interactions between nucleic acid base layers of DNA and photochromic dyes which are intercalated into DNA double helical structures. It was reported in 2005 that photochromic dyes such as spiropyran derivatives were intercalated into DNA-lipid complex films to attain a high speed optical switching and a high density data storages. Most of optical dyes are water-insoluble and soluble in organic solvents, so that DNA-lipid complexes which are soluble in polar organic solvents were used to intercalate these water-insoluble optical dyes.

DNA molecules can form cross-linking bridges among DNA chains by multi-cations such as water-soluble polyamine cations. So, it is highly expected that polyamine cations may form direct cross-linking with DNA molecules in water solutions in the presence of water-soluble dyes to form films or fibers by ion cross-linking reaction together with doping. This simultaneous doping and cross-linking methods will be very much useful for device preparations.

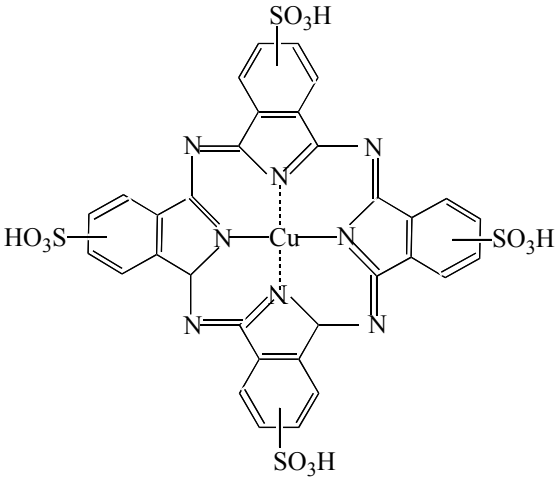
Based on this expectation, water-soluble polyamine cations as shown below were used for the *in-situ* crosslinking of DNA in the presence of water-soluble optical dyes. Pure DNA was dissolved in an amount of 1g per 100ml of pure water and the aqueous solution of DNA was cast on a Teflon plate to evaporate water and a clear film of DNA was obtained. The DNA film was dipped into aqueous solution of polyamine cations as shown below:



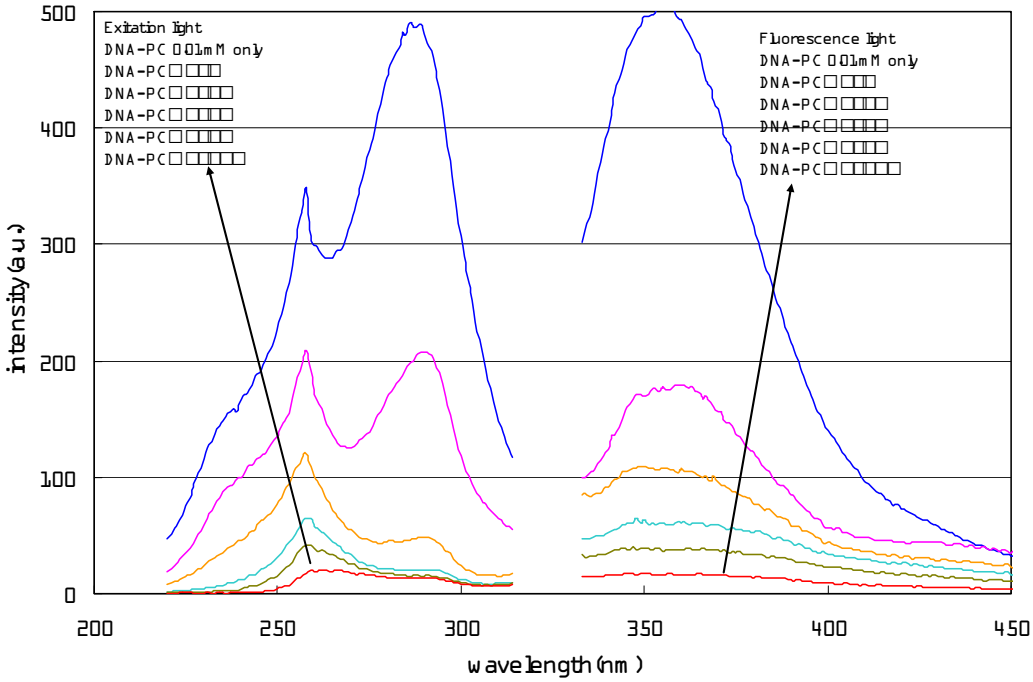
Clear and transparent films of DNA which were insoluble in water were easily obtained.

Phtalocyanines are well known photoresponsive dyes and they can form various complexes with metal ions such as Cupper or Tatanium ions. These metal-phtalocyanine complexes have various photo-activities to enhance photovoltaic behaviors. So, water-soluble sulfonated cupper phtharocyanine dye(Cu-PC) was used for the *in-situ* intercalation into crosslinked DNA films. The structure of sulfonated cupper phtharocyanine dye is shown below:

Tetrasulfonated cupper phthalocyanine



Aqueous solutions of DNA and the cupper phtharocyanine（Cu-PC） of each different concentrations were irradiated by UV light and fluorescence light emission was measured by a fluorescence spectrometer and results are shown in Figure 1.



and

Fig.1　Exitation and fluorescence spetcra of DNA-Cu-PC in water

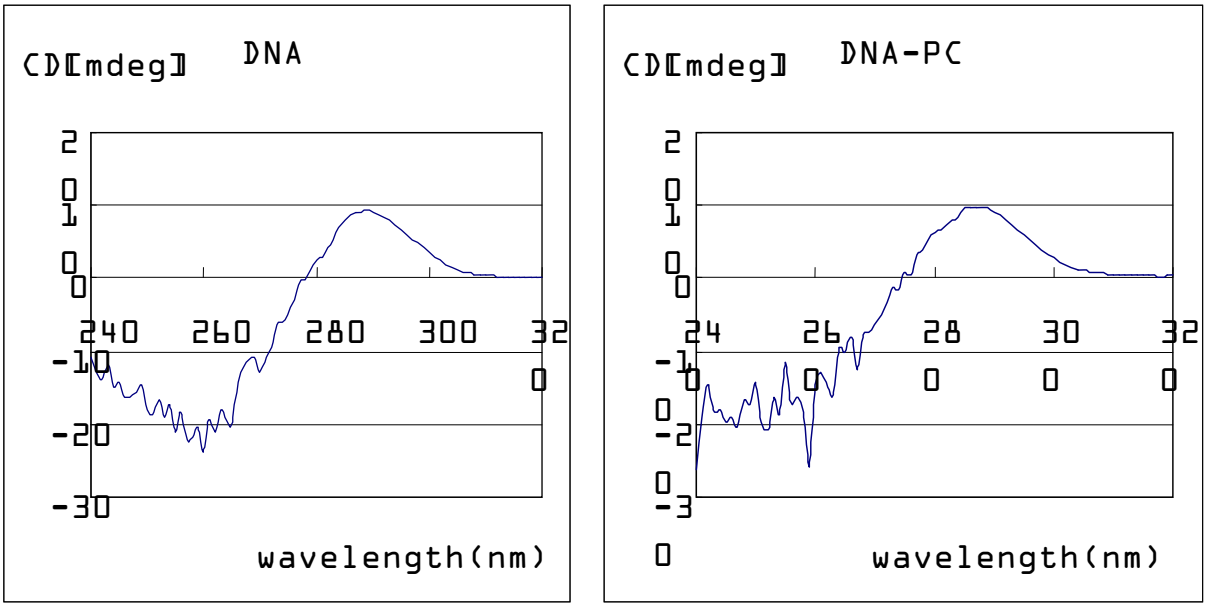


Fig. 2　CD spectra of DNA and DNA in the presence of Cu-PC in water

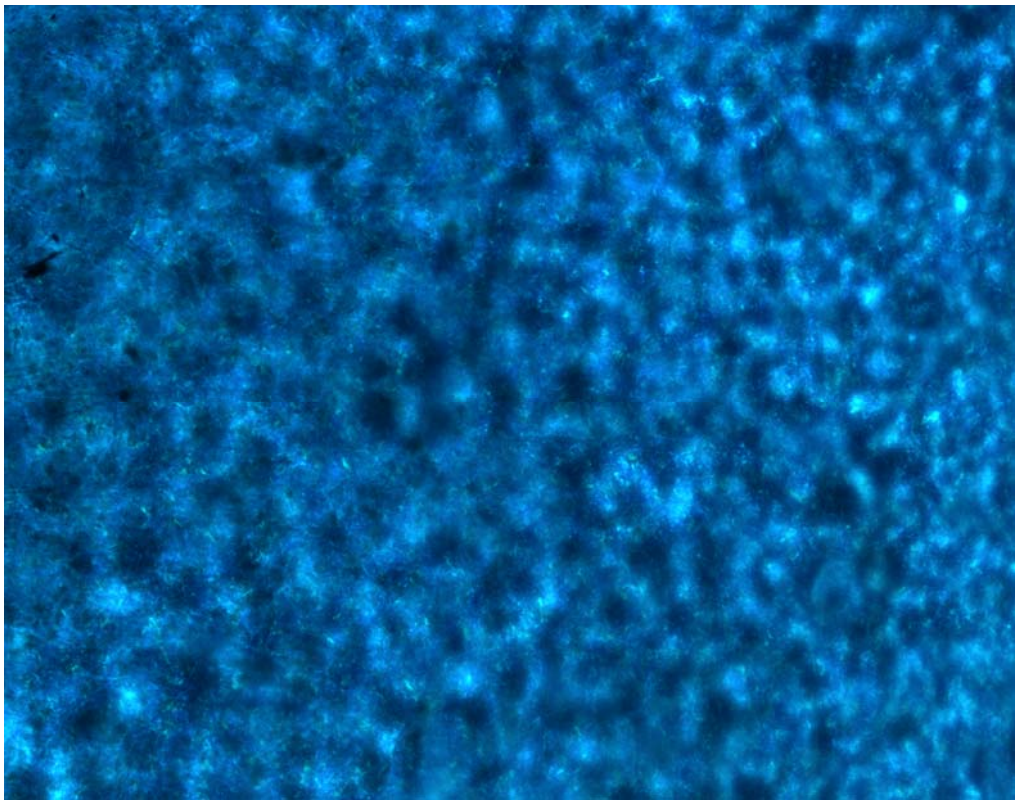


Fig. 3　Surface structure of DNA-Cu-PC film irradiated by UV light

The *in-situ* film formation of DNA with polyamine complexes was carried out in the presence of Cu-PC in water and a blue film was obtained.　Surface picture of the DNA-Cu-PC film which was

irradiated by UV light is shown in Figure 3, which showed a little aggregation of Cu-PC in the DNA film. However, dispersion states of Cu-PC look almost homogeneous and uniform, so Cu-PC is homogeneously dissolved in DNA

Refractive indices of the DNA-Cu-PC films were measure as shown in Figure 4 which indicates refractive index changes before and after UV irradiation.

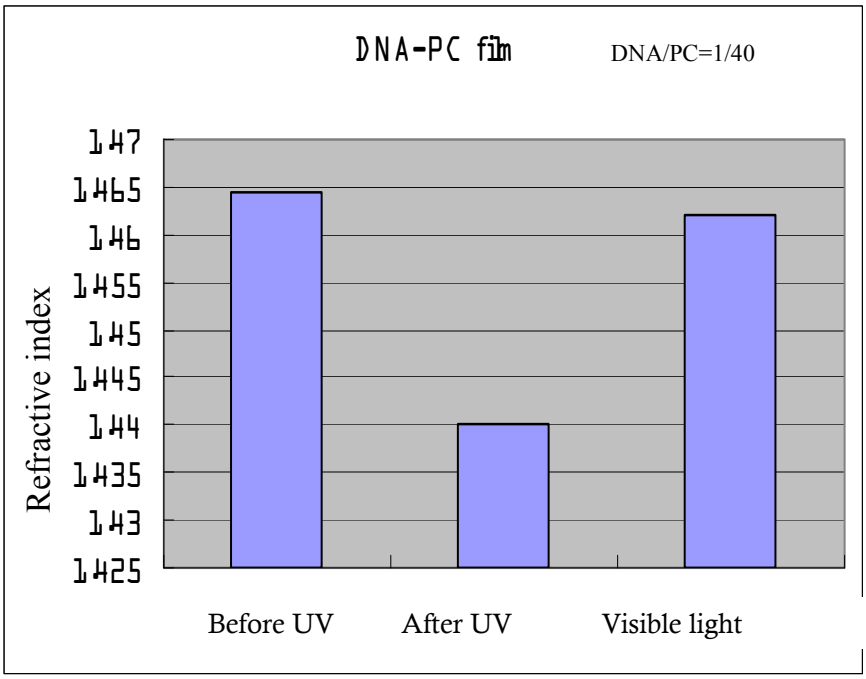


Fig.4 Refractive index changes before and after UV irradiation, followed by visible light irradiation

Figure 4 indicates that the refractive index of the DNA-Cu-PC film changed after UV irradiation, while it goes back again to almost the same refractive index by irradiating visible light. These results suggest that the DNA-Cu-PC film which was obtained by the *in-situ* crosslinking and doping by Cu-PC, could be applied as erasable memory devices.

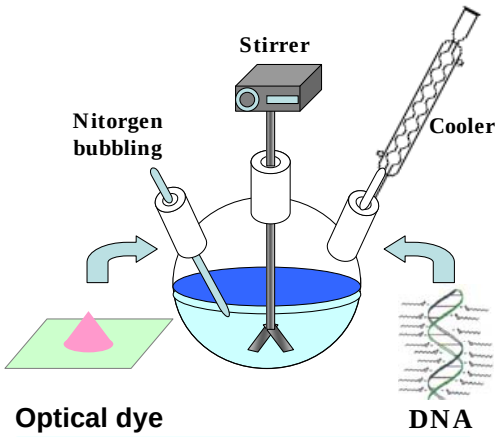
Research 3 Nano-particles derived from DNA-polymer Hybride Materials Containing Optical Dyes

Nano-particles are quite interesting for optical applications as light is confined in the nano-particles to enhance fluorescence light by a resonance effect. So, Nano-particles derived from DNA-polymer hybrids containing optical dyes were prepared.

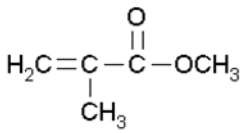
Nano-particles of various diameters from 100nm to 1μ derived from DNA-polymer hybrid materials were prepared by an emulsion or suspension polymerization of monomers such as methyl methacrylate (MMA) in the presence of DNA in water by a conventional radical polymerization or low-temperature plasma polymerization methods. DNA molecules were confined in the nano-particles. Intercalation of optical dyes is carried out by dipping the nano-particles containing DNA into dye solutions. Optical properties of the dye-intercalated nano-partcles are characterized in terms of fluorescence intensity enhancement, amplified spontaneous emission (ASE) of light and lasing effect.

Suspension polymerization of methylmethacrylate (MMA) was carried out in an aqueous solution containing DNA in the presence of poly(vinylalcohol) as a stabilizer for the suspentsion as shown below:

DNA containing nano-particles by suspension polymerization

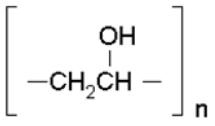


Monomer



Methyl methacrylate

Stabilizer



Poly(vinyl alcohol)

Polymerization conditions

Stirring speed 400rpm Temp. 60

Initiator $K_2S_2O_4$

Particle sizes could be controlled from 15~100 μm by changing concentration of MMA monomer and stirring speed.

Fig. 1 shows enlarged picture of the obtained particles of PMMA containing DNA and dye (DAMSDPPB). Fig. 2 indicate flurescence emission from the particle.

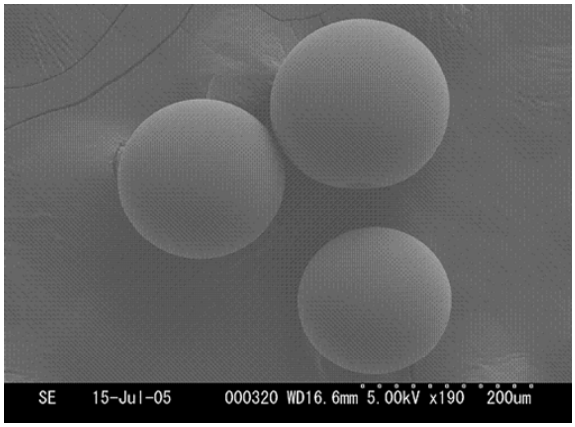


Fig.1 Picture of the particles containing DNA and dye

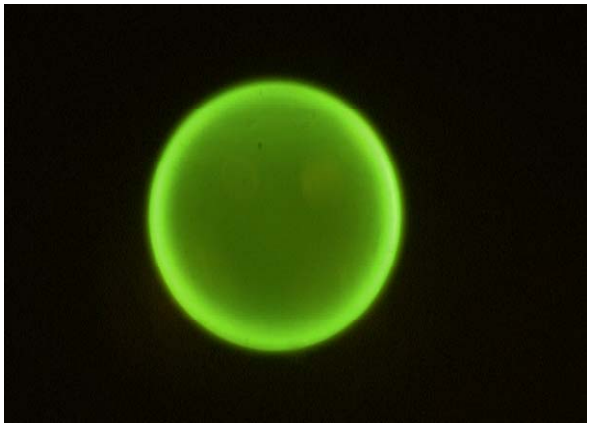


Fig.2 Fluorescence ffrom the particle

Particle size:~15μm

Circular dichroism (CD) spectra were measured for the particles in order to confirm the DNA existence in the particles and the double helical structure of DNA and results are shown in Fig. 3 and 4 ,which can confirm the existence of DNA with the double helical structure, as Fig. 4 indicates an essential Cotton effect which is similar as Fig. 3 of DNA-Na.

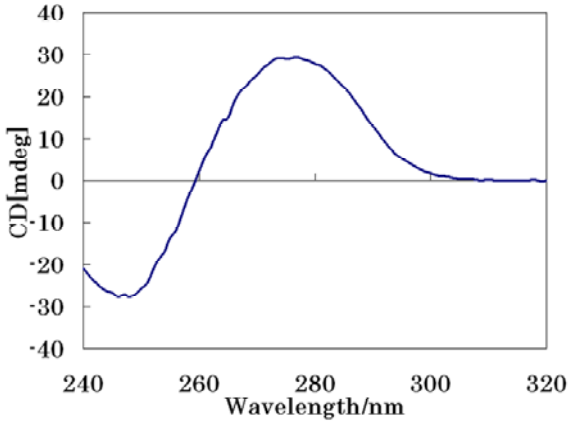


Fig.3 CD spectrum of DNA-Na

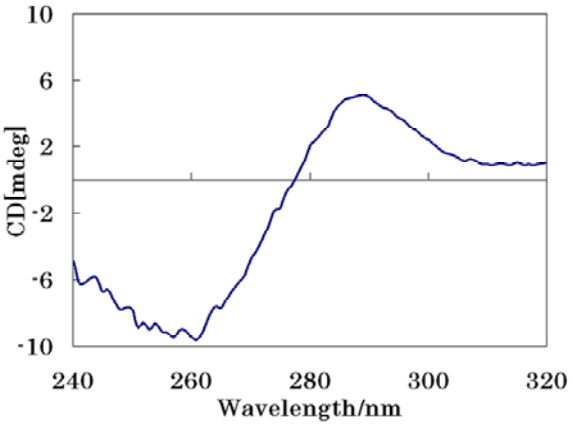


Fig.4 CD spectrum of DNA-lipid in the particle

Optical properties of the particles containing DNA and dye were investigated by an experimental setup to measure an emission spectrum of fluorescence from the dyes in the particles as shown in Fig. 5 and results are shown in Fig. 6.

It is seen in Fig. 6 that the DNA-containing particles sowed much narrowing spectrum of fluorescence light with threshold value of 1mJ/cm^2, while no narrowing of the spectrum occurred for the no-DNA particles without any threshold value. Therefore, it is clear that the DNA-containing particles enhanced fluorescence light of the dye owing to the intercalation of the dye into DNA molecules.

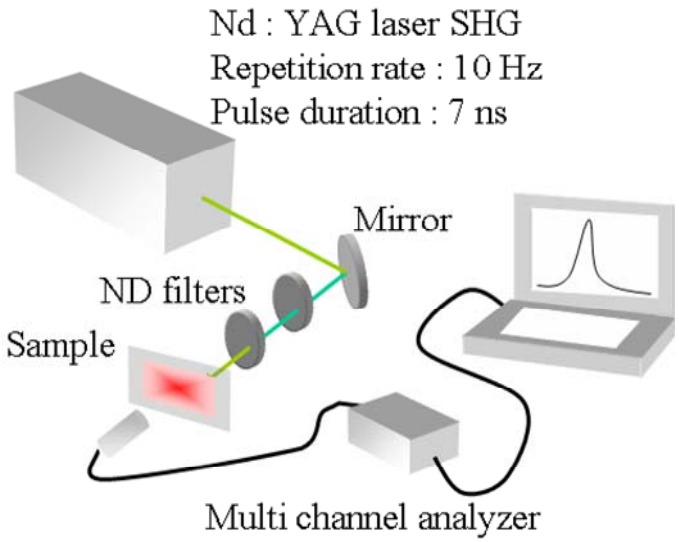


Fig. 5 Experimental setup for the measurement of emission spectra

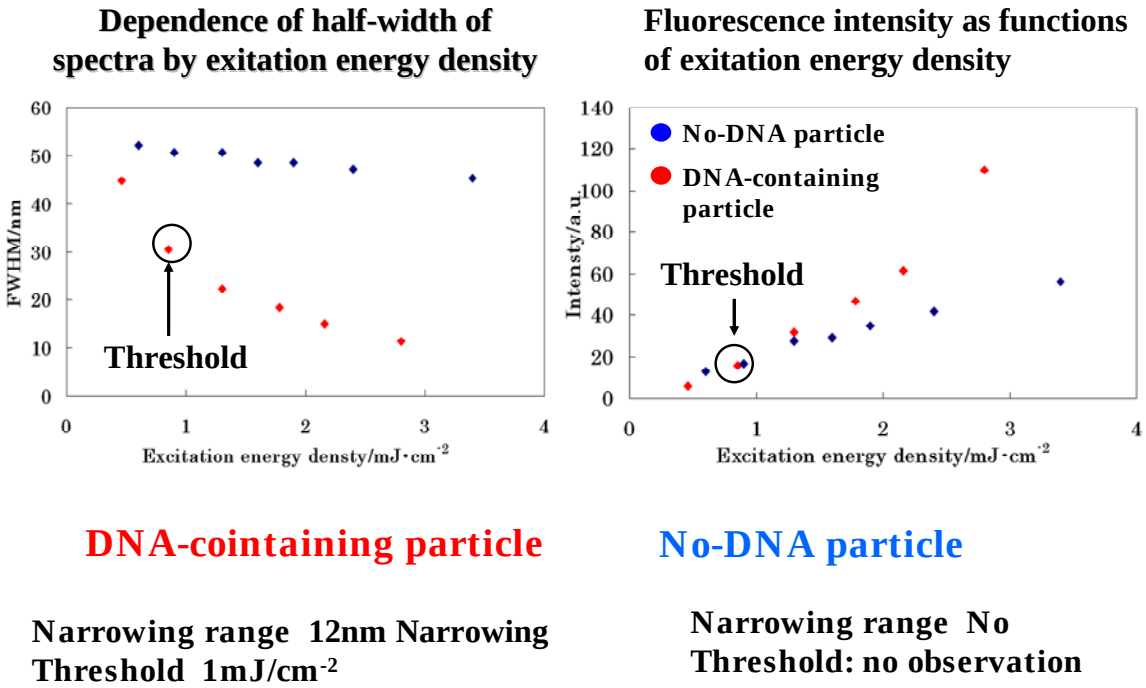


Fig. 6 Comparison of optical properties between DNA-containing and no-DNA particles

Optical properties of DNA-containing particle and film were compared in terms of threshold values of half-width narrowing spectra and narrowing ranges as shown in Fig. 7, which indicates that the DNA-containing particles showed much narrowing of spectrum width with a threshold value of 1 mJ/cm^{2,} while the narrowing range of the half-width of the DNA-containing film was around 40 nm and no threshold value was observed. Therefore, the DNA-containing particles enhanced fluorescence light much better than film, possibly owing to light enhancement by a resonance effect within the nano-particles

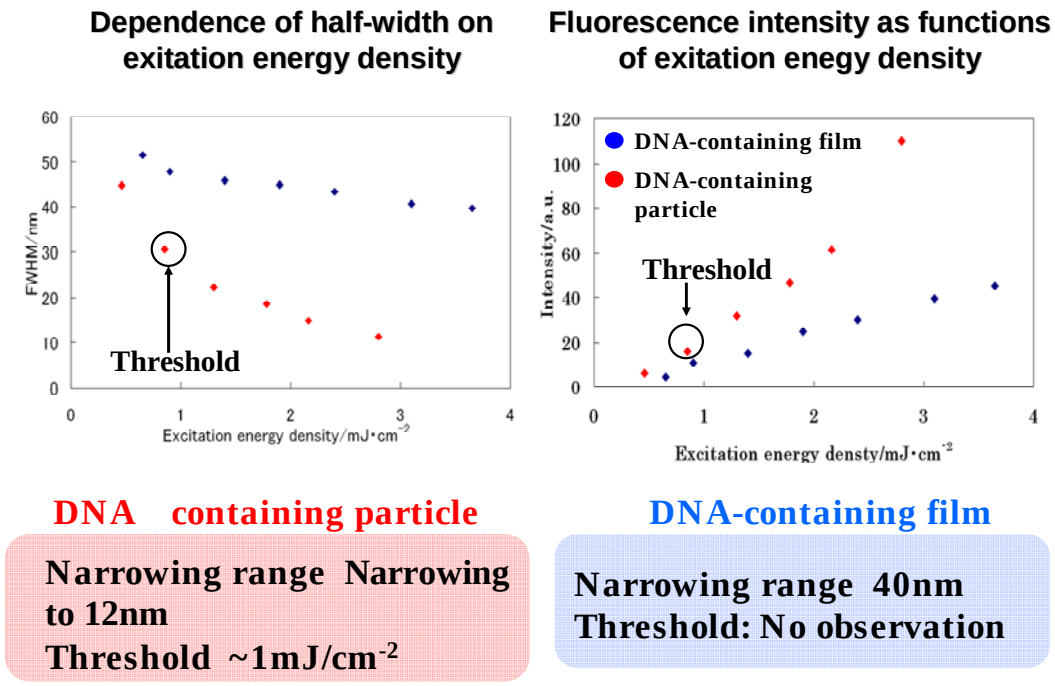


Fig. 7 Comparison of optical properties between DNA-containing particle and film.

Research 4　Design and demonstration of high density data storage system

High density data storage system was designed to enhance photochromic responces in DNA wave guide which had a grating structure by using refractive resonances through the wave guide. The design is schematically shown in Figure 1 where the film of DNA-CTMA of refractive index of 1.493~1.495 is overcoated with DNA-CBDA which is cetyl benzyl dimethylammonium lipid., having refractive idex of 1.493~1.495.

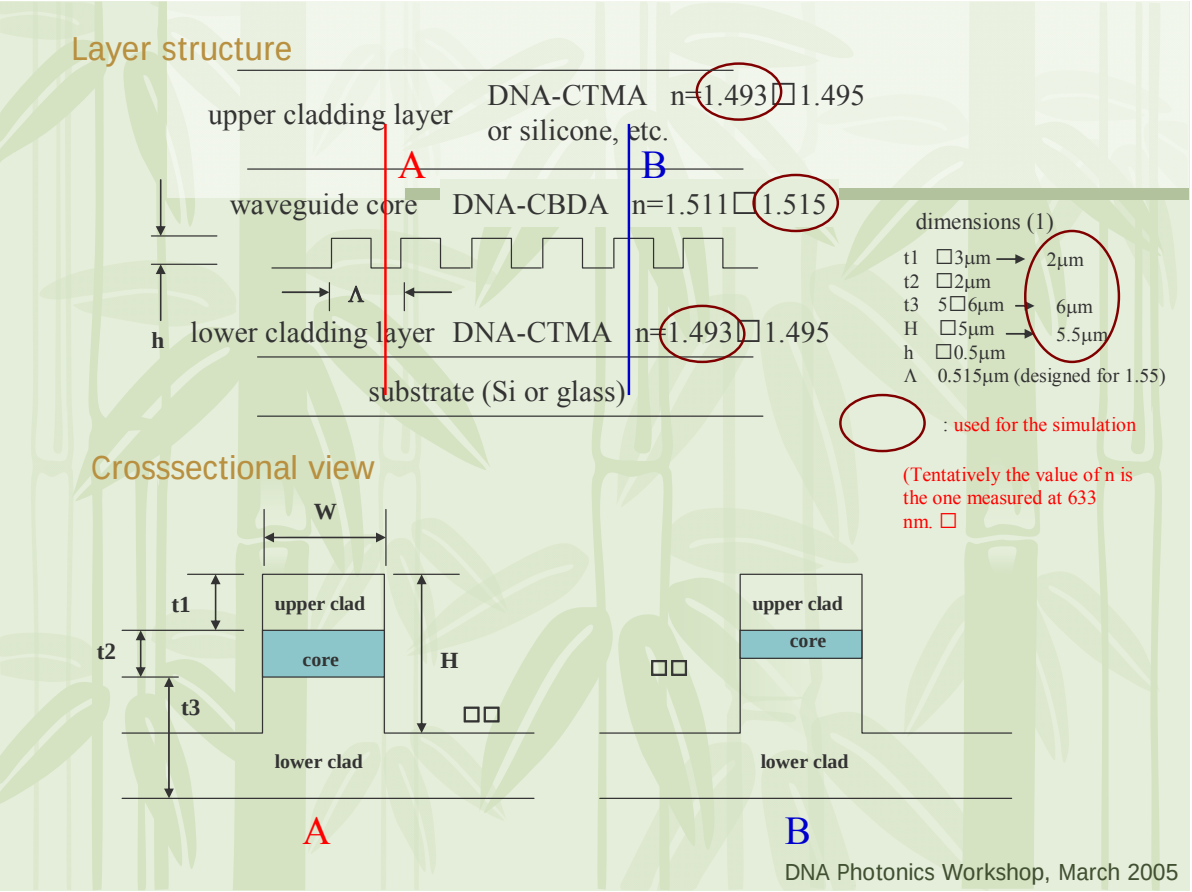


Fig. 1 Design of grating wave guide

Simulation of refractive changes of the DNA grid suggests a possible design for the rapid response of the photochromic reactions of various dyes.　Based on this simulation, DNA grid wave guide was fabricated by irradiating the DNA-CTMA thin film coated on a Si wafer, as shown in Figure 2 for the process.

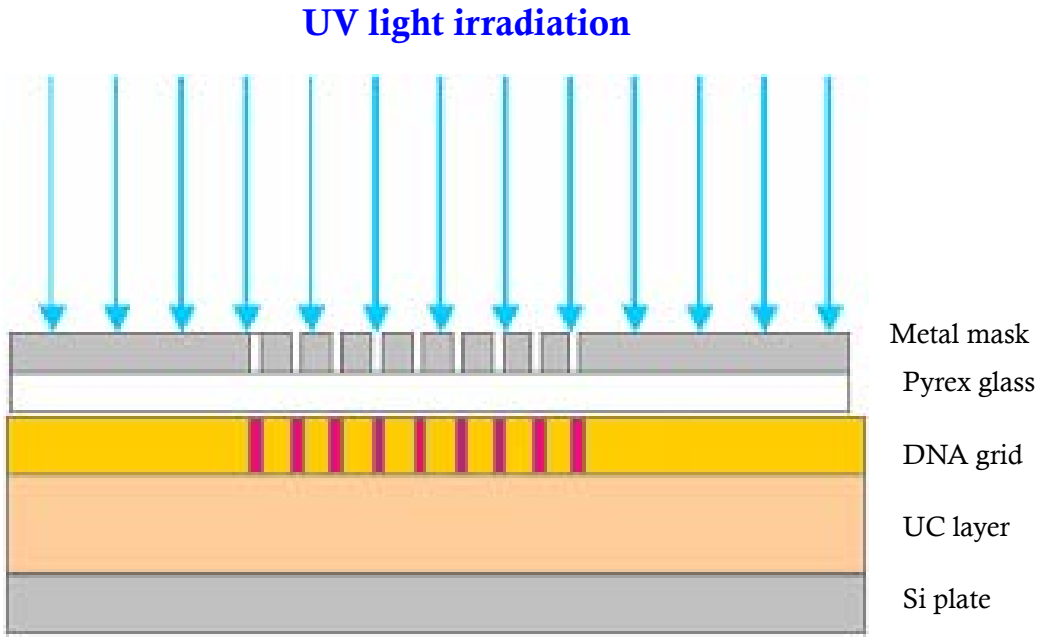


Figure 2　Process to prepare DNA grid of long period

Patterns of the DNA grid with long periods of 50 and 70 μm which were prepared by this process are shown in Figure 3 which indicates that the patters were prepared as expected.

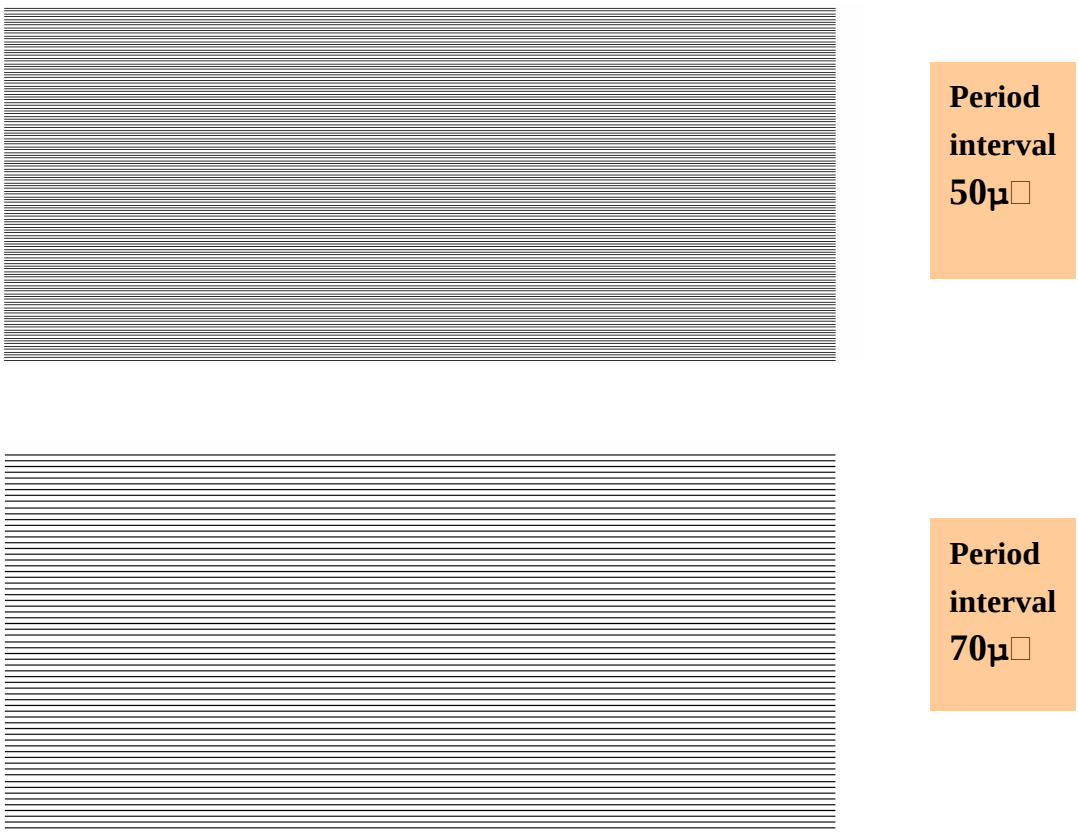


Figure 3　Fabricated patters of DNA grid

Figure 4 shows the DNA device outlook for practical applications.

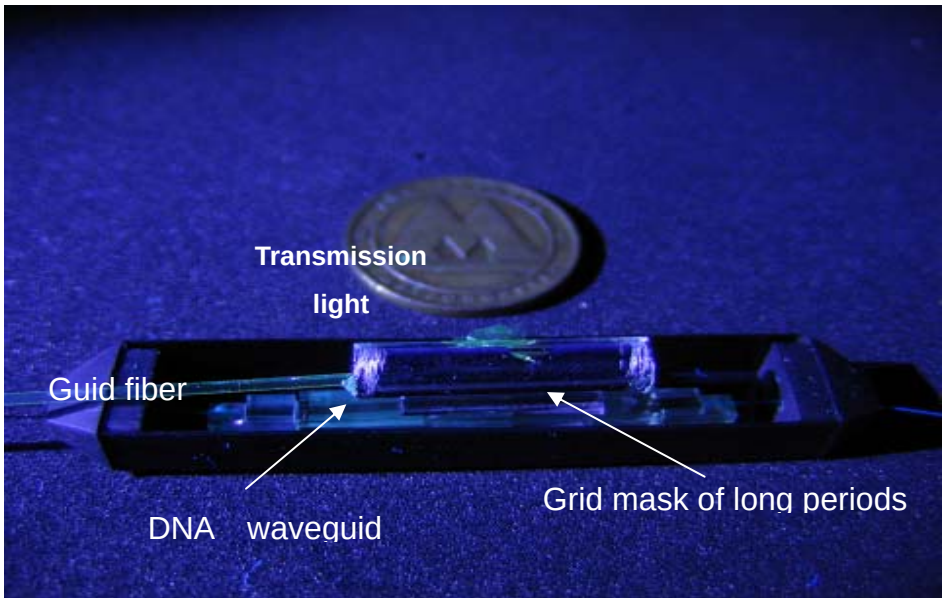


Figure 4　Outlook of the DNA grid waveguide

Figure 5 shows results of light reflection by using the DNA switching device and a very sharp reflection at 1560.5nm which is expected by the simulation. This result suggest practical applications of the DNA optical device. However, a life time to keep the good performance of the DNA device is not sufficient and a good sealing of the DNA device to keep out moisture or oxygen is necessary.

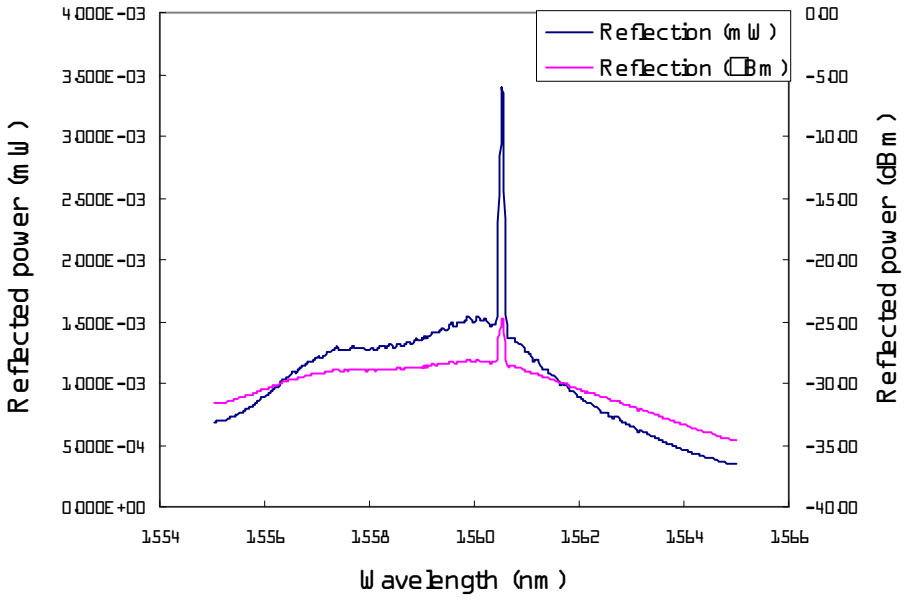


Figure 5　Reflection pattern of the DNA grid-type waveguide

Workplan in 2006 and 20007

2006

1. Intercalation of Photochromic Dyes with DNA

Intercalation of DNA by various photochromic dyes into double helix of DNA is expected to enhance and amplify light owing to electron-electron interactions between nucleic acid base layers of DNA and photochromic dyes which are intercalated into DNA double helical structures. Appropriate photochromic dyes such as azobenzene derivatives are searched in order to attain high density data storages into the dye-intercalated DNA films.

DNA molecules can form cross-linking bridges among DNA chains by multi-cations such as polyamine cations. So, it is highly expected that polyamine cations may form direct cross-linking with DNA molecules in water solutions in the presence of dyes to form films or fibers by ion cross-linking reaction together with doping. This simultaneous doping and cross-linking methods will be very much useful for device preparations.

2. Complexation of DNA by Phthalocyanine-metal Compounds

Phthalocyanines are well known photoresponsive dyes and they can form various complexes with metal ions such as Cupper or Tatanium ions. These metal-phthalocyanine complexes have various photo-activities to enhance photovoltaic behaviors. Since double helical DNA molecules can form a stable intercalation with polyaromatic compounds and also can form metal complexes, it is highly expected that these metal-phthalocyanine compounds are intercalated into DNA molecules so that high density data storages are possible by intercalating metal-phthalocyanine compounds with DNA under irradiation of UV and visible light.

3. Characterization of DNA structure after Intercalation with Optical Dyes and Phthalocyanine Compounds

Structural characterization of intercalated DNA molecules with optical dyes or phthalocyanine compounds is carried out by spectroscopic (CD, UV-visible spetra, IR and NMR) and scanning probe microscopic (SPM) methods.

4. Nano-particles derived from DNA-polymer Hybride Materials Containing Optical Dyes

Nano-particles of various diameters from 100nm to 1μ derived from DNA-polymer hybrid materials are prepared by an emulsion or suspension polymerization of monomers such as styrene and methyl methacrylate in the presence of DNA in water by a conventional radical polymerization or low-temperature plasma polymerization methods. DNA molecules are included within the nano-particles. Intercalation of optical dyes is carried out by dipping the nano-particles containing DNA. Optical properties of the dye-intercalated nano-partcles are characterized in terms of fluorescence intensity enhancement, amplified spontaneous emission (ASE) of light and

lasing effect.

5. Developments of High Density Data Storage Devices

High density data storage devices which are derived from dye-intercalated DNA and nano-particles containing DNA-dye are prepared to form films and coating on various plates. Photo-responsive speeds and reading-out capacities are investigated by irradiating UV and visible light by means of sharp spot laser beams.

Research Milestone

1. FY 2006 (January-December)

Photochromic dyes such as various azobenzene derivatives and metal phthalocyanine compounds are intercalated into DNA-lipid complexes. Optimum reaction conditions are determined in terms of temperature, concentration or solvent effects to form intercalated DNA films. Good solvents for the DNA complexes are searched for fabrications as uniform films.

Various metal-phthalocyanine compounds are investigated by using various center metal ions such as Cupper, Tatanium or novel metals for the intercalation into DNA molecules.

Nano-partices derived from DNA-polymer hybride materials are prepared by an emulsion polymerization of monomers such as styrene and methyl methacrylate in the presence of DNA in water and dye intercalation is carried out by dipping the nano-particles in dye solutions

2. FY 2007 January-December

Structure-property relations of intercalated DNA-lipid complexes are elucidated by spectroscopic and scanning probe microscopic (SPM) methods. Characterization of dye-and metal-phthalocyanine-intercalated DNA is carried out to determine interactions of dye and metal-phtalocyanines with DNA molecules. Photo-responses of the nano-particles containing DNA-dye are investigated in terms of light amplification and data storage capacities.

Fabrications of the intercalated DNA-lipid complexes and the nano-particles containing DNA-dye are carried out to prepare thin films and optical responses and data storage capacities are investigated by irradiating sharp spot UV and visible laser light. Device systems for high density data storage are designed to established for practical applications as molecular devices.

Date: March 1, 2006

Signed by Naoya Ogata